Characterizing Methods of Measuring Flow-Mediated Dilation in the Brachial Artery

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Regulation of vascular tone is one of the many important functions of the vascular endothelium. Endothelial dysfunction is a critical early event in the pathogenesis of atherosclerosis and occurs in the absence of angiographic disease. Flow-Mediated Dilation (FMD) is a noninvasive technique commonly used to evaluate endothelium-dependent vasodilation in humans and gauge the health of the cardiovascular system. Reductions in brachial artery FMD have been strongly correlated with disease progression and are predictive of future cardiac events. The flow stimulus for brachial artery FMD occurs as a result of the increased shear stress following deflation of an occlusion cuff around the upper arm. Using 2-dimensional ultrasound, changes in arterial diameter up to 5-minutes following cuff deflation are calculated from baseline image measurements. Along with pulsed Doppler measures of flow velocity through the artery, flow-mediated, endothelium-dependent vasodilation can be assessed. There is debate among investigators, however, about the proper positioning of the occlusion cuff during FMD testing. It is thought that placement of the cuff around the upper arm may not accurately reflect the impact of nitric oxide, a critically important molecule released as a result of the increased shear stress created by the FMD technique. Data suggest that the production of other endogenous metabolites may also contribute to FMD-related changes when positioning the cuff around the upper arm. To overcome the potential influence of such molecules, researchers now suggest that the occlusion cuff be placed below the elbow allowing a more precise estimate of nitric oxide mediated dilation. The purpose of this study is to compare the differences in FMD between the two methodologies of occlusion cuff placement. In addition, this study will determine the method that is easier for ultrasound technicians to perform and will produce a low coefficient of variance between technicians. Ultimately, the results of this study will help in tracking any adverse cardiovascular effects of spaceflight.

Nomenclature

NO = nitric oxide
ISS = International Space Station
JSC = Johnson Space Center

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FMD = flow mediated dilation
eNOS = endothelial nitric oxide synthase
ANOVA = analysis of variance
CPHS = Committee for the Protection of Human Subjects
USRP = Undergraduate Student Research Program

I. Introduction

The vascular endothelium is responsible for vasomotor regulation and the maintenance of vascular homeostasis which includes monitoring fibrinolysis, thrombosis and angiogenesis [1,2,3]. The vascular endothelium produces a number of factors that contribute to the equilibrium between vasodilation and vasoconstriction. One of the most important factors is thought to be nitric oxide (NO). When these functions are not maintained in equilibrium, because of reduced NO production, the endothelium is considered to be dysfunctional. Mechanisms that contribute to dysfunction include oxidative stress, which may lead to the onset of atherosclerosis [4]. Therefore, reduced levels of NO production are pertinent in the early detection of adverse cardiovascular events. Exposure to a microgravity habitat threatens cardiovascular and, more specifically, arterial health. Long-duration flights going to the International Space Station (ISS) present factors that have been shown to contribute to vascular dysfunction, such as decreases in blood flow, which result in a decrease in the release of NO. Endothelial dysfunction post flight and the cardiac risks that can result are important aspects of spaceflight being studied at Johnson Space Center (JSC). The Cardiovascular Laboratory at JSC has therefore proposed a noninvasive method to measure called flow mediated dilation (FMD)endothelial dysfunction and thus better monitor cardiovascular health of astronauts on long-duration flights.

FMD is a noninvasive technique used to evaluate endothelium-dependent vasodilation. Blood vessels respond to physical and chemical stimuli that occur in the lumen. In addition, blood vessels have the ability to regulate tone, adjust blood flow and manage the distribution of arterial pressure [4]. Measures of FMD are results of shear stress produced by a hyperemic state. As blood rushes back through the arteries it is applying a tangential force on the artery walls, known as shear stress. The shear stress activates the channel which allows calcium to enter the cell. The calcium is required to activate enzyme Nitric Oxide Synthase (eNOS) within the endothelial cell which leads to NO production from L-arginine. NO is secreted by endothelial cells to the smooth cells and is responsible for vasodilation [5,6]. The amount an artery dilates corresponds to the health of the artery and indicates how well the artery is functioning. A large vasodilation correlates with a healthy and functioning endothelium [3]. Deleterious changes in the brachial artery strongly correlate with coronary endothelial dysfunction and risks of cardiac diseases [7]. The brachial artery is observed during an FMD evaluation.

An FMD test begins with a flow measurement, via ultrasound, through the brachial artery followed by the placement and inflation of an occlusion cuff at a specified location on the arm. The cuff occludes blood flow and creates an ischemic environment. The cuff remains inflated for 5 minutes [8]. Hyperemic response follows with the deflation of the cuff. Figure 1 depicts the two cuff placements; placement A is at the upper arm proximal to the elbow and B is at the forearm distal to the elbow. The ultrasound image acquisition occurs in the same spot on the brachial artery for both occlusion methods. Placement A has been used in the past but some researchers think it may not accurately account for the changes in levels of NO and of other molecules, such as metabolites, that interfere in the FMD measurements [5,6]. The purpose of this study is to characterize the difference between two methods of inducing FMD in the brachial artery. Placement B has become a method of interest because data suggest that at this location FMD better reflects NO- mediated dilation, and metabolites will not have significant influence. Placement A has been said to be an uncomfortable location to occlude, and, at this location, it is hard to get a good image of the brachial artery because of possible collapse. However, placement B has none of these disadvantages. It is expected that there will be a greater change in FMD measurements and larger vasodilation in the upper arm, placement A, than at the forearm, placement B. However, this study could also show that there are no major differences between these placements of the cuffs.
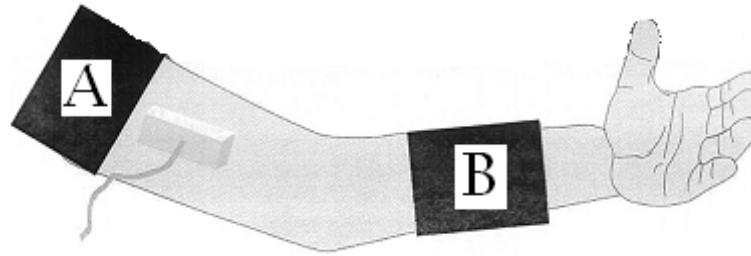
Figure 1. Ultrasound of Brachial Artery FMD Using Two Methods [8]
Depiction of cuff placement at the upper arm, A, and cuff placement at the lower arm, B, while taking an ultrasound image of the brachial artery.

II. Materials and Methods

A. Subjects

Subjects were recruited through the Human Test Subject Facility at JSC, and all subjects passed a modified Class III Air Force physical. Five men and 5 women were chosen and all were approved by the Committee for the Protection of Human Subjects (CPHS). Participants arrived at the Cardiovascular Laboratory at JSC, and studies lasted a total of 54 min. Subjects were asked to lie supine.

B. Study Protocol

1. Test Constraints

Subjects were asked to refrain from the following as they are factors that can affect FMD measurements: no heavy meal within the preceding 4 hours, a light snack of complex carbohydrates within the preceding 2 hours, no caffeine, nicotine or alcohol within the preceding 12 hours and no medications. The study was conducted in a quiet, temperature controlled quiet room.

C. Equipment

The following were used to conduct an evaluation of FMD: a Dinamap blood pressure cuff, a Hokanson inflatable cuff to occlude the brachial artery (see Figure 1 A), a CX50 ultrasound machine (Philips Medical, Bothell, WA) with a 12-4 mmHg linear phased-array probe and a Fisher Scientific timer.

D. Image Acquisition

Two-dimensional (2D) ultrasound images of the brachial artery of the right arm were obtained with the arm supinated and abducted ~45°. Pulsed Doppler data were also obtained to evaluate brachial blood flow characteristics at baseline and after cuff release. The subject's blood pressure was obtained on the left arm. With the subject supine, a 12-4 mmHg linear phased-array ultrasound probe was positioned longitudinally at the site. After 2D and Doppler baseline data were acquired, the cuff was inflated to 70 mmHg above the systolic blood pressure for 5 minutes. Upon cuff release, pulsed-wave Doppler data were obtained at 10 and 20 seconds. Two-dimensional images of the brachial artery were obtained at baseline, then at 30 seconds, then every 15 seconds until 3 minutes after deflation. The brachial artery diameter was also monitored at minutes 4 and 5. Images were stored digitally for subsequent off-line analysis.

E. Artery Measurements

Methods of measuring FMD, either placement A or B, were randomly chosen before the start of the test. A Hokanson cuff was placed on the right upper arm and connected to a rapid cuff inflator. Prior to inflation, baseline measurements of the brachial artery were obtained and recorded using the ultrasound equipment. The Hokanson cuff was then inflated to 70 mmHg over subject's systolic pressure. The cuff remained inflated for 5 minutes. This created an ischemic environment in the artery. The cuff was then rapidly deflated resulting in reactive hyperemia

and vasodilatation. Images were taken throughout the following 5 minutes after release. There was a 15-minute rest period before FMD measurements were collected using the second method.

F. Statistical Analysis

A two-way repeated-measures analysis of variance (ANOVA) was performed to compare baseline and peak measurements, in both groups. A two tailed pair wise *t*-test was run to support the results and conclusions of the percentage change from baseline to peak measurements. A *P*-value of less than 0.05 was considered statistically significant. Measurements were analyzed twice and at random by one analyzer. A quality control value of greater than 10 was considered to have poor reproducibility of analysis. An intraobserver coefficient of variation of 0.01 was calculated. All data presented in using mean ± standard error.

III.   Results

A. **Participant Demographics**

A total of 10 subjects, 5 male and 5 female, all healthy adults, were initially chosen at random for this study. Two subjects were excluded because of technically inadequate scans, leaving a total of 8 subjects, 4 male and 4 female.

B. Comparisons of FMD Measurements and Methods

Consent was given for the use of images. Figure 2a is a representative baseline ultrasound image. Figures 2b-e are ultrasound images and data graphs that are representative of a response post occlusion. The ultrasound images provide a visual representation of the brachial artery as well as how the diameter was measured.
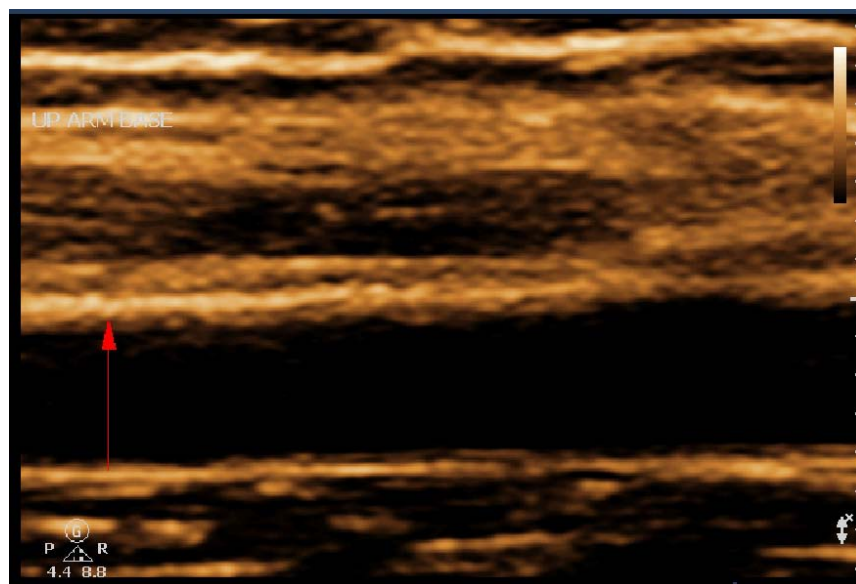
Figure 2a. Baseline Image *Baseline measurement of brachial artery. Red arrow indicates baseline diameter of brachial artery*
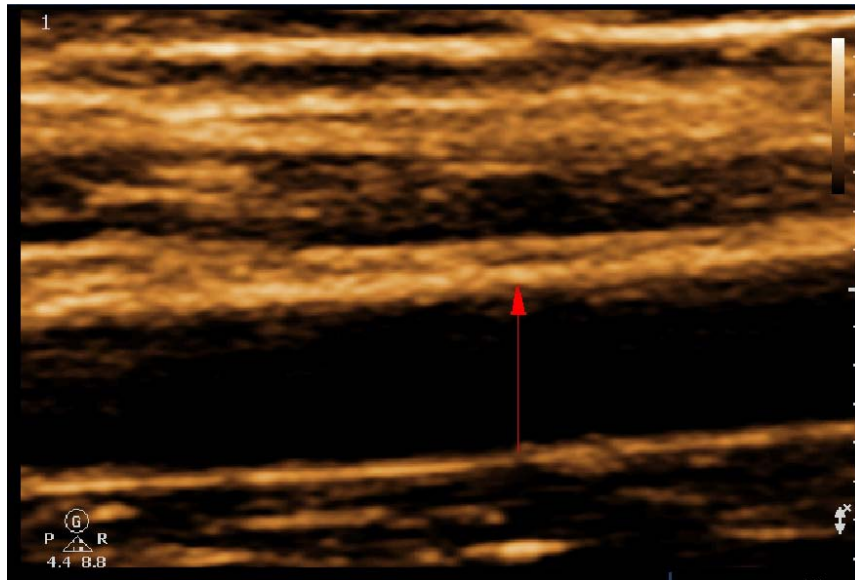
Figure 2b. Image after Deflation with Cuff at Upper Arm *Measurement of brachial artery 1 min after deflation with occlusion cuff at upper arm. Red arrow indicates diameter of brachial artery after 1 min of reactive hyperemia*
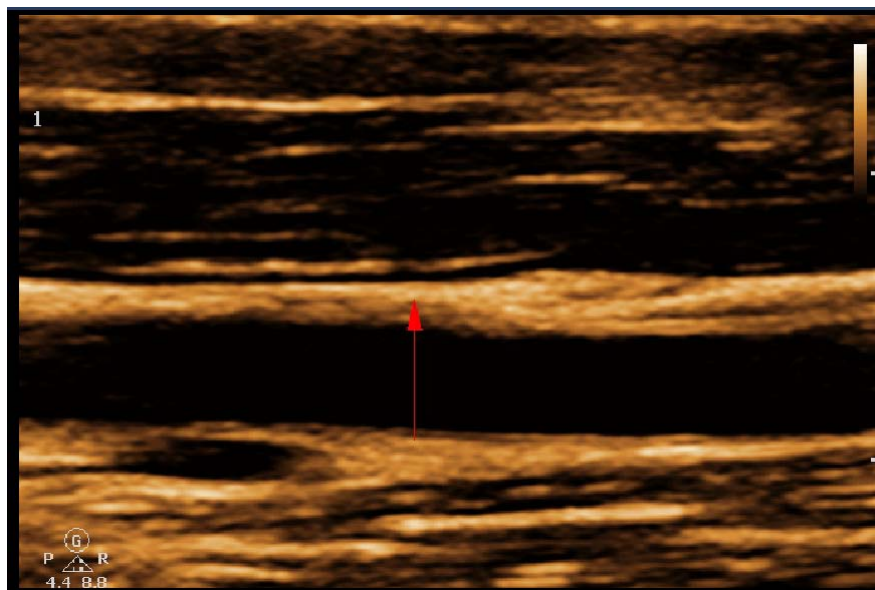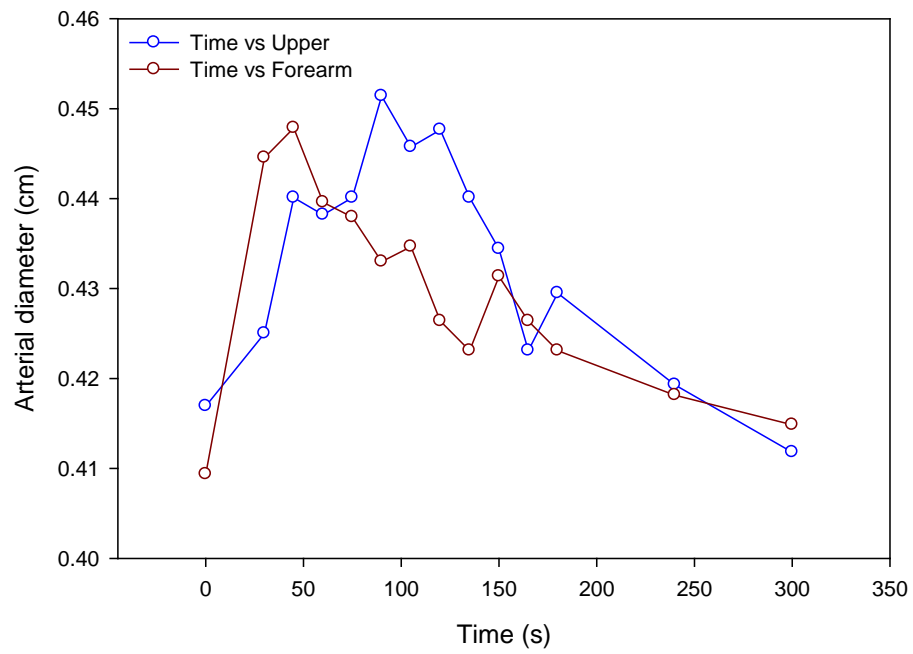

Figure 2c. Image after Deflation with Cuff at Forearm *Measurement of brachial artery 1 min after deflation with occlusion cuff at forearm. Red arrow indicates diameter of brachial artery after 1 min of reactive hyperemia*

Figure 2d. Change in Artery Diameter *Graphical representation of one subject's change in artery diameter over time.*
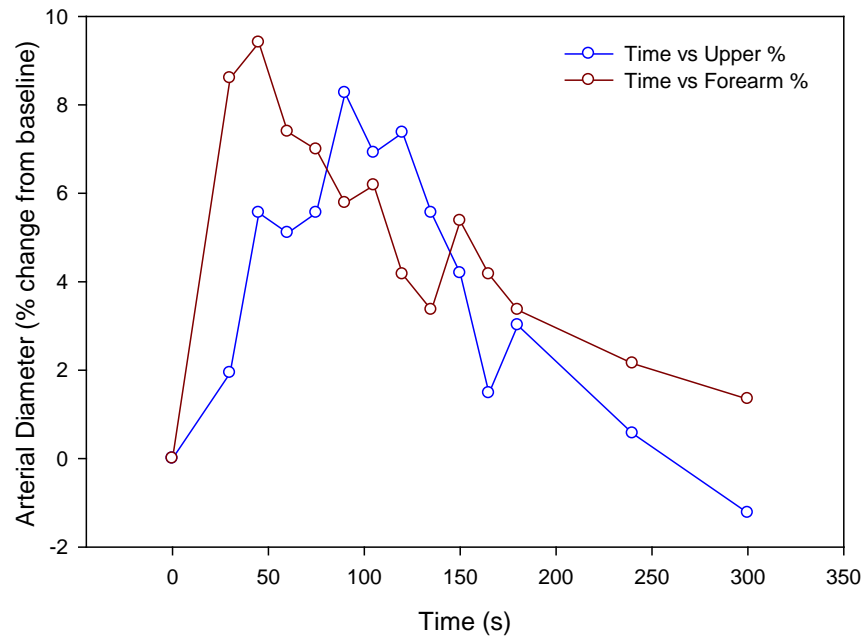
Figure 2e. Percentage Change in Artery Diameter *Graphical representation of one subject's percentage change in artery diameter over time.*

Figure 3 is a graphical representation of average diameter measurement as a function of time for both upper arm and forearm. The upper arm and forearm show a similar trend with no statistically significant differences found.
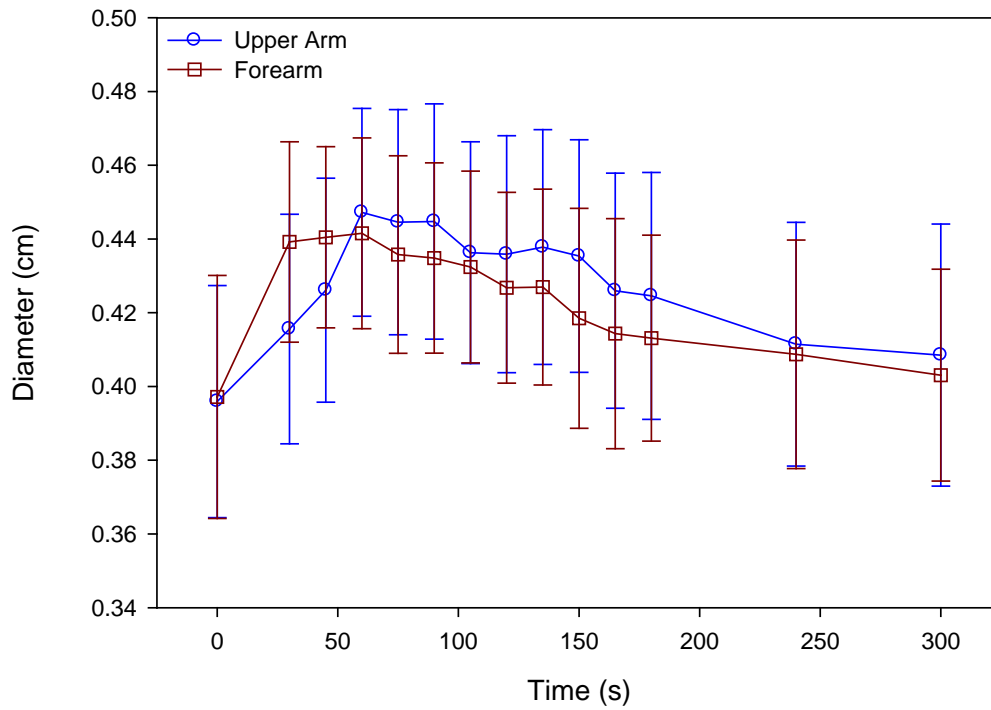
Figure 3. Mean Changes in Artery Diameter *Graphical representation of change in group mean artery diameters over time for both upper arm and forearm.*

Figure 4 is a graphical representation of average percentage change from baseline measurements to peak measurements for both upper arm and forearm. The upper arm and forearm show a similar trend.
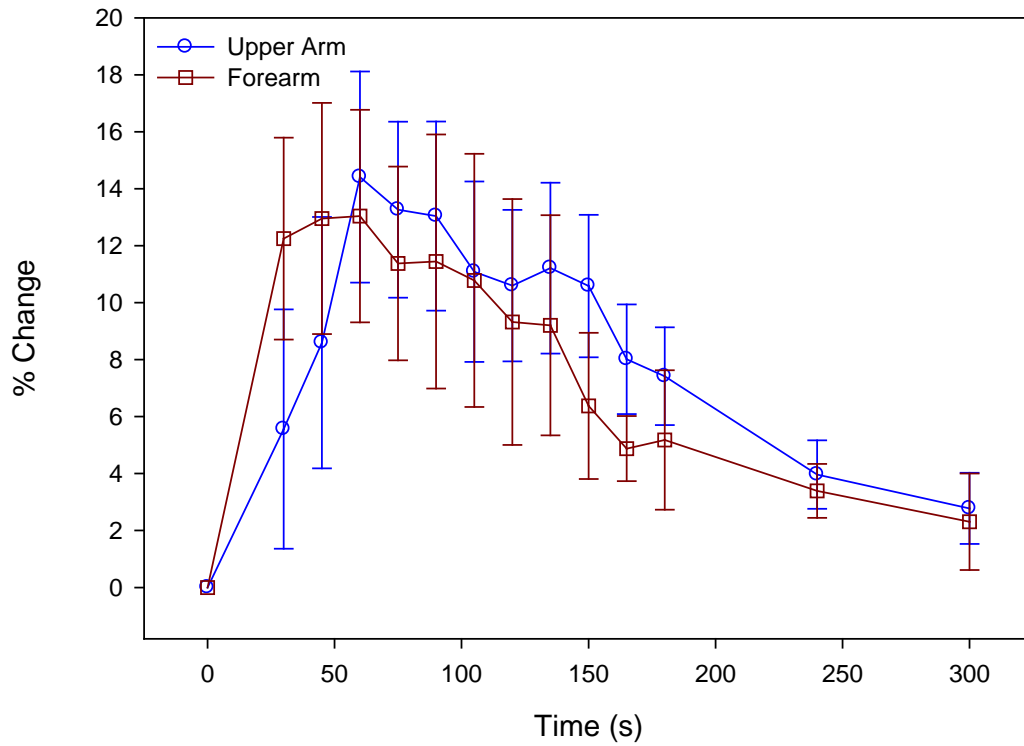
Figure 4. Mean Percentage Change in Artery Diameter *Graphical representation of percentage change in group mean response of artery diameters over time for both upper arm and forearm*
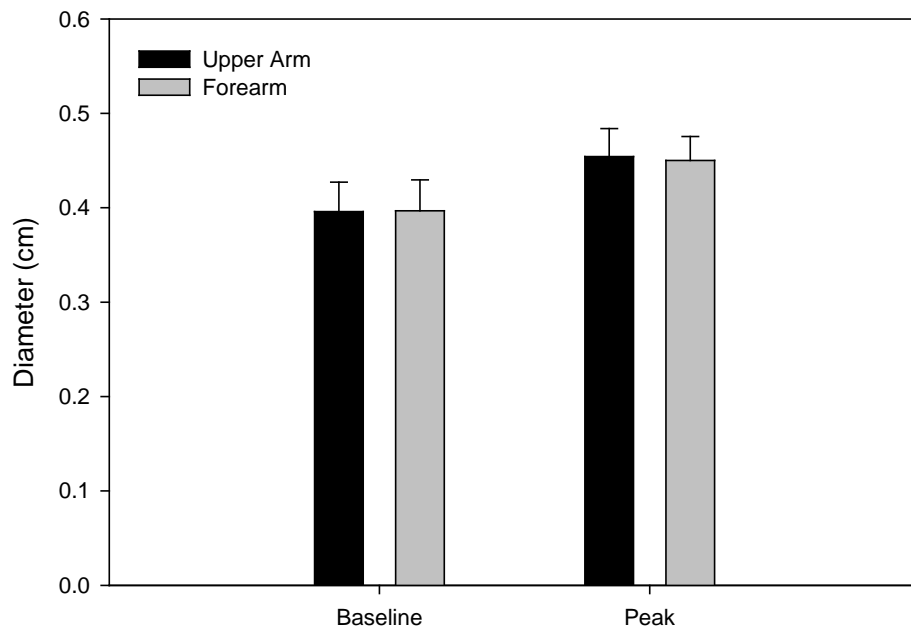
Figure 5. Mean Baseline and Peak *Graphical representation of mean baseline and peak for both upper arm and forearm.*
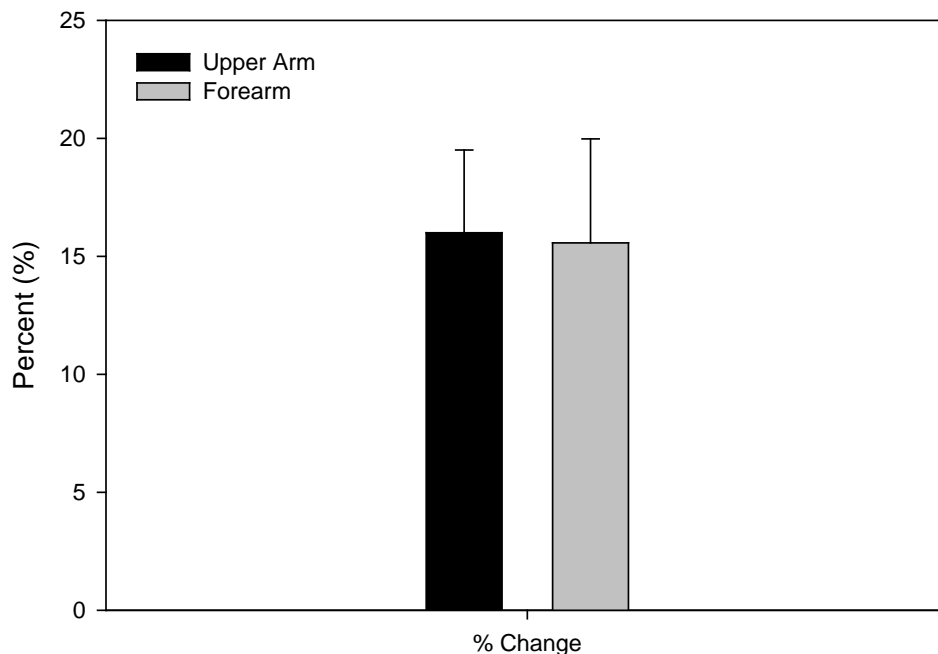
Figure 6. Mean Percentage Change *Graphical representation of mean percentage change for both upper arm and forearm from baseline measurements to peak measurements.*

IV.   Discussion

The purpose of this study was to characterize the difference, if any, between two methods of inducing Flow-Mediated Dilation in the brachial artery. It is important to notice that the error bars at the peak points for the upper arm and the forearm support the fact that there is no significant difference of when the peak diameter occurred for each method. There can be several reasons as to why the error bars on figure 3 are so large, such as differences between men and women, physical fitness and a very small sample size.

Figure 5 and a supporting two-way repeated-measures ANOVA indicated that there is no significant difference between baseline measurements at the upper arm and at the forearm as well as no significant difference between peak measurements at the upper arm and at the forearm.

Figure 6 and a supporting pair wise *t*-test indicated that there was no significant difference between the percentage change, from baseline to peak measurements, at the upper arm and the percentage change found at the forearm. The Cardiovascular Laboratory at JSC has placed the occlusion cuff at the upper arm in the past but has become interested in switching methods because of the many advantages that come with placement B (see figure 1). The results from this study serve as the statistical support to change measuring methods.

V.   Conclusion

The results of this study allow members of the Cardiovascular Laboratory at JSC to switch methods of measuring FMD without the risk of losing valuable information. Placement of an occlusion cuff at the forearm presents attractive advantages over placement at the upper arm. Some of those advantages are a more comfortable experience for the subject, an easier acquisition for ultrasound technicians and a better representation of nitric oxide production. This method of measuring FMD will allow the endothelial health of astronauts to continue to be monitored for the purpose of predicting cardiac events and the overall maintenance of the cardiovascular system of astronauts on long-duration flights.

Acknowledgments

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